ORIGINAL ARTICLE

Comparison of antimicrobial, larvicidal and anthelmintic activity of *Zingiber officinale* Rose. cow urine extract

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Abstract

Background: Cow urine is believed to have therapeutic value and used in many drug formulations. It has been used along with herbs to treat various ailments by traditional healers. **Aim**: The present study was aimed to evaluate and compare the antimicrobial, larvicidal, and anthelmintic activity of *Zingiber officinale* Rose. (ZO) with cow urine extracts. **Materials and Methods:** The antimicrobial activity was performed using five microbial strains, namely, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, and *Aspergillus flavus*; larvicidal activity was evaluated using 3rd and 4th instar stage larvae; and anthelmintic activity using red earthworms (*Lumbricus rubellus*) for raw and photoactivated cow urine. **Results:** Antimicrobial activity of ZO photoactivated cow urine extract (ZOPUE) at 20 µg/ml concentration was found to show better minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (MIC: 13.8 µg/ml for *E. coli*, MBC value >25 µg/ml) when compared with ZO raw cow urine extract (ZOUE) (MIC: 14.3 µg/ml for *E. coli*, MBC value >25 µg/ml). Larvicidal activity of ZOPUE at a concentration of 25% v/v showed a better mortality rate of 38.6% v/v when compared with ZOUE at a concentration of 25% v/v as 35.2% v/v. Anthelmintic activity of ZOPUE was found to show better paralysis and death rate at a concentration of 10 mg/ml, that is, 38.2 min for paralysis and 58.9 min when compared with ZOUE, that is, 39.6 min paralysis rate at a concentration of 10 µg/ml and death time observed was 59 min at 10 µg/ml concentration. **Conclusion:** Antimicrobial, larvicidal, and anthelmintic activity of ZOPUE were more significant when compared with ZO extract prepared with raw cow urine.

Key words: Anthelmintic, antimicrobial, cow urine, larvicidal, *Zingiber officinale* Rose.

INTRODUCTION

Cow is equated to mother in Indian tradition and her urine is considered as panacea of all diseases.[1] In substrata, several medicinal properties of cow's urine are mentioned and reported to be efficient in weight loss, reversal of certain cardiac and kidney problems, indigestion, stomach ache, edema, etc. Cow urine has a unique identity in Ayurveda and has been described in "Sushruta Samhita" and "Ashtanga Sangraha" to be the most effective substance secretion of animal origin with innumerable therapeutic values. It has been recognized as water of life or "Amrita" (beverages of immortality), the nectar of God in Vedas. In India, drinking of cow's urine has been practiced for thousands of years. Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow, namely, urine, dung, milk, ghee, and curd. A number of formulations mentioned in Ayurveda describe the use of Panchagavya components either alone or in combination with drugs of herbal, animal, or mineral.[2]

Cow urine is a divine medicine and is used for the treatment of diabetes, blood pressure, asthma, psoriasis, eczema, heart attack, blockage in arteries, fits, cancer, piles, prostrate, arthritis, migraine, thyroid, ulcer, acidity, constipation, and gynecological problems. It is also used to increase the

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nitrogen content of the soil, for better rearing of honeybees, hasten the pubertal age of the heifers exposed to bull's urine, and as pesticide and larvicide for the fodder crops. Cow urine contains all substances, which are naturally present in the human body. Thus, the consumption of cow's urine maintains the balance of these substances and helps in the treatment of incurable diseases. It is natural, eco-friendly with no residual effects, economical, easily available, and may be harnessed as a potential therapeutic agent.[1]

The distillate of cow's urine is an efficient bioactivity enhancer and availability facilitator for bioactive molecules (antibiotic, antifungal, and anticancer drugs). The distillate of cow's urine helps in absorption of antibiotic across the cell membrane in animal cells, gram-positive and gram-negative bacteria at 40-50°C, and transport across the gut wall by two to seven times. It also increases the activity of gonadotropin-releasing hormone (glutathione) conjugate with bovine serum albumin (GnRH-BSA) and zinc. The GnRH-BSA conjugate is known to show deleterious effects on reproductive hormones and estrous cycles of female mice. Cow urine has been granted US Patents (No. 6,896,907; 6,410,059 and 6,410,059) for its medicinal properties. "Panchgavya" is also used as fertilizers and pesticides in agricultural operations. In recent studies, cow urine has been proved to be an effective pest controller and larvicide when used alone and also in combination with different plant preparations by enhancing the efficacy of different herbal preparations.[3] Cow urine has been reported to boost the annual ryegrass yield by enhancing nitrogen component of the soil and a marked depression in N_2 fixation 10% annually in clovers.[4] Scientists in Uttarakhand are making use of cow urine to save bees from microbial diseases during their rearing process. Cow urine facilitated rapid and holistic recovery in disease-infected combs, promoted the growth of brood, enhanced the efficiency of worker bees in the colonies, and thus revealed that the cow urine may serve as a potential eco-friendly measure for the management of European foulbrood, a serious bacterial disease of honeybee brood found throughout the world in honeybee colonies. It has an indirect control of mite diseases in colonies too.[5]

Cow urine is basically an excellent germicide, a potent antibiotic, and a bioenhancer.[6] Bioenhancers are chemical entities which promote and augment the bioavailability of the drugs which are mixed with them and do not exhibit synergistic effect with the drug. The various bioenhancers available are piperine, garlic, *Carum carvi*, *Cuminum cyminum*, lysergol, naringin, quercetin, niaziridin, glycyrrhizin, stevia, and cow urine distillate ginger. Out of these, *C. cyminum* and niaziridin are the potential bioenhancers of future.[7] Cow urine not only enhances the effect of above mentioned drug but also reduced the toxic and other adverse effect of synthetic drugs. As per Ayurveda, cow urine is required to purify and detoxify many crude drugs. Cow ghee processed with herbals known as "ghrita" has central nervous system activity, immunomodulatory activity, hepatoprotective effect, wound-healing activity, sedative, and anticonvulsant activity. Cow urine distillate known as "Kamdhenu ark" exhibited many biological activities including immunomodulatory potential, antimicrobial effect, etc.[8] Ginger (*Zingiber officinale* Rose. [ZO], *Zingiberaceae*) extracts have been extensively studied for a broad range of biological activities including antibacterial, anticonvulsant, analgesic, antiulcer, gastric antisecretory, antitumor, antifungal, antispasmodic, antithrombotic, hypocholesterolemic, antiallergic, antiserotonergic, anticholinergic, and other beneficial activities. Many studies have proved that ginger is endowed with strong antioxidant, antigen toxic, antimutagenic, and anticarcinogenic properties.[9]

In view of above potential activities of cow urine and ZO, we have evaluated and compared antimicrobial, larvicidal, and anthelmintic activity of ZO raw cow urine extract (ZOUE) and ZO photoactivated cow urine extract (ZOPUE).

MATERIALS AND METHODS

Collection and Authentication of Drug

ZO was collected from local market in Rohtak. The drug was authenticated by Dr. Sunita Garg, NISCAIR and the

Table 1: MIC and MBC of ZOUE and ZOPUE against microbial strains

Microbial strains	ZOUE		ZOPUE	
	MIC (µg/ml)	MBC/MFC (µg/ml)	MIC (µg/ml)	MBC/MFC (µg/ml)
Bacterial				
E. coli	14.3	>25	13.8	>25
S. aureus	7.6	25	11.7	>25
B. subtilius	14.9	25	9.6	25
Fungal				
A. niger	11.9	>30	10.6	>30
A. flavus	10.8	30	11.4	>30

E. coli: Escherichia coli, S. auerus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, MFC: Minimum fungicidal concentration, ZOUE: *Zingiber officinale* raw cow urine extract, ZOPUE: *Zingiber officinale* photoactivated cow urine extract

Table 2: Zone of inhibition (mm) of different microbial strains at various concentrations of ZOUE and ZOPUE and standard CPF and FLZ

Extract	Concentration (µg/ml)	Zone of inhibition (mm) Mean±SD									
		E. coli		*S. aureus*		*B. subtilis*		*A. niger*		*A. flavus*	
		Extract	CPF	Extract	CPF	Extract	CPF	Extract	FLZ	Extract	FLZ
ZOUE	20	12.6±0.25	25±0.25	12.9±0.25	25±0.1	14.0±0.5	25±0.1	14.8±0.25	30±0.10	14.9±0.25	25±0.25
	10	12.3±0.11	25±0.5	12.5±0.5	25±0.5	13.9±0.25	30±0.25	13.4±0.5	30±0.5	14.7±0.25	30±0.5
	8	11.8±0.25	30±0.5	11.9±0.25	30±0.25	13.7±0.50	25±0.5	12.8±0.25	25±0.5	13.7±0.5	25±0.25
	4	10.7±0.5	25±0.25	10.7±0.5	25±0.5	12.7±0.25	25±0.25	11.9±0.25	30±0.25	12.3±0.25	30±0.10
	2	9.7±0.10	30±0.25	9.7±0.1	30±0.25	11.9±0.3	30±0.5	11.8±0.10	25±0.5	11.9±0.10	25±0.25
ZOPUE	20	12.9±0.25	30±0.25	13.8±0.25	25±0.1	14.8±0.5	25±0.1	15.2±0.25	30±0.10	15.1±0.25	25±0.25
	10	12.2±0.11	25±0.5	13.4±0.5	30±0.5	14.2±0.25	30±0.25	14.8±0.5	30±0.5	14.8±0.25	30±0.5
	8	11.9±0.25	30±0.5	12.6±0.25	30±0.25	13.9±0.50	25±0.5	14.4±0.25	25±0.5	14.1±0.5	25±0.25
	4	11.5±0.5	20±0.25	11.5±0.5	25±0.5	13.2±0.25	25±0.25	13.7±0.25	30±0.25	13.8±0.25	30±0.10
	2	10.8±0.10	30±0.25	10.7±0.1	30±0.25	12.8±0.3	30±0.5	13.4±0.10	25±0.5	13.5±0.10	25±0.25

CPF: Ciprofloxacin, FLZ: Fluconazole, ZOUE: *Zingiber officinale* raw cow urine extract, ZOPUE: *Zingiber officinale* photoactivated cow urine extract, SD: Standard deviation

voucher specimen number for ZO is NISCAIR/RHMD/Consult/2014/2557-136-2. The standard drugs used for antimicrobial studies were ciprofloxacin and fluconazole obtained as gift sample from Dr. Morepen Laboratories, Parwanoo.

Photoactivation of Cow Urine

The cow urine used in this study was photoactivated by maintaining it in sunlight for 72 h in a transparent glass beaker. Then, cow urine was filtered to free it from debris and precipitated materials.

Processing and Extraction of Crude Drug

ZO was coarsely powdered and extracts of ZO with raw cow urine (ZOUE) and photoactivated cow urine (ZOPUE) were prepared by maceration technique and stored in refrigerator for further use.

Test Microorganisms

Tested bacterial strains were *Escherichia coli* NCIM 2931, *Staphylococcus aureus* NCIM 2079, and *Bacillus subtilis* NCIM 2063 and fungal strains, namely, *Aspergillus niger* NCIM 2957 and *Aspergillus flavus* NCIM 2027.

Turbidimetric Method

The turbidimetric method or tube dilution method was used for determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC). ZOUE and ZOPUE were serially diluted to give a concentration of 20, 10, 8, 4, and 2 µg/ml in test tubes containing 1 ml sterile nutrient broth. These tubes were inoculated with 100 µl of bacterial suspension in saline and incubated at 37°C for 24 h (for plates containing bacterial cultures), 37°C for 2 days (for plates containing *A. flavus* culture), and 25°C for 7 days (for plates containing *A. niger* culture). A tube containing nutrient broth only was seeded with the test organism to serve as a control. All the tubes were then incubated at 37°C for 24 h and examined for growth by observing turbidity. The MBC of the plant extract on the clinical bacterial isolates was carried out by pipetting out 0.1 ml bacterial culture from the mixture obtained in the determination of MIC tubes which did not show any growth and subcultured on to nutrient media and incubated at 37°C for 24 h. After incubation, the concentration at which there was no single colony of bacteria was taken as MBC.

Paper Disc Diffusion Method

To determine the antibacterial activity of the crude extract, 0.2 µl individual bacterial and fungal cultures were poured

Table 3: Percentage mortality rate of larvae after 24 and 48 h with ZOUE and ZOPUE

Extract	Concentration (%v/v)	Mortality after 24 h (%v/v)	Mortality after 48 h (%v/v)	Standard after 24 h (%v/v)	Standard after 48 h (%v/v)
ZOUE	25	35.2	60.2	38.7	67.6
	50	39.3	62.5	45.8	72.3
	75	40.4	63.6	49.7	78.9
	100	45.9	68.9	53.5	82.7
ZOPUE	25	38.6	65.5	41.3	63.7
	50	42.2	68.9	43.6	65.7
	75	46.4	75.5	46.8	68.9
	100	49.9	78.9	49.8	72.8

ZOUE: *Zingiber officinale* raw cow urine extract, ZOPUE: *Zingiber officinale* photoactivated cow urine extract

Table 4: Paralysis time and death time of earthworms with ZOUE and ZOPUE

Extract	Concentration (µg/ml)	Paralysis time (in min)	Death time (in min)	Paralysis time (in min) standard	Death time (in min) standard
ZOUE	10	39.6	59.0	42.7	64.4
	25	42.8	68.8	46.9	71.4
	50	56.9	75.9	59.9	79.9
	75	60.8	79.6	67.9	84.5
	100	67.8	83.05	75.6	86.9
ZOPUE	10	38.2	58.9	41.8	64.5
	25	41.9	62.6	45.9	65.9
	50	56.0	68.6	59.8	72.5
	75	63.9	75.9	65.7	79.6
	100	65.5	79.7	71.8	82.9

ZOUE: *Zingiber officinale* raw cow urine extract, ZOPUE: *Zingiber officinale* photoactivated cow urine extract

into nutrient agar medium (30 ml) in petri plates (90 mm). Sterilized filter paper discs (Whatman No. 1; 6 mm in diameter) soaked in different beakers containing the dissolved extracts of different concentrations were taken out with sterilized forceps and air-dried and placed on plates with the different organisms. The plates were incubated at 37°C for 24 h for bacterial strains and for 7 days at 25°C for *A. niger* and *A. flavus*. After incubation, the inoculated plates were observed for zones of inhibition in millimeter diameter using a transparent ruler.[10]

Preparation of Test Concentrations

Four test concentrations (25, 50, 75, and 100 µg/ml) were prepared through single dilution method and stored in labeled specimen bottles for larvicidal bioassay. The standard solution was prepared by mixing of polysorbate and acetone (6:4).

Source of Larvae

The 3rd and 4th instar larvae were collected in April 2015 from the Institute of Horticulture Technology, Greater Noida (Uttar Pradesh), Delhi, NCR.

Larvicidal Bioassay

A total of 10 actively swimming larvae (2nd and 3rd instars) were released into 25 ml capacity beakers containing 16 ml of each larvicide extract solutions in different concentrations (25-100% v/v). A set of control using 2% dimethyl sulfoxide as Control 1 and untreated sets of larvae in (tap) water as Control 2 were run for comparison. The beakers were then stored for 24-48 h. Results were obtained after 24 h and 48 h.

Red earthworms (*L. rubellus*) were collected from Shree Ladwa Gaushala, Ladwa and the standard drug (albendazole) was taken from Dr. Morepen Laboratories, Parwanoo.

Test Organisms for Anthelmintic Activity

Indian adult earthworms (*L. rubellus)* were taken and washed with normal saline. The earthworms were used for experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

Anthelmintic Activity

Albendazole was used as a standard drug. All the extracts and drug solutions were freshly prepared. Five groups with six earthworms in each were placed in the prepared extract and five control groups were placed in albendazole. Observations were made for the time until the paralysis and death of an individual worm. The paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors.

RESULTS

The antimicrobial activity evaluated for *Z. officinalis* (ZO) was found to be more in ZOPUE when compared with ZOUE as shown in Table 1. Antimicrobial study of ZO was further supported by paper disc diffusion method, in which significant antimicrobial activity was noted in ZOPUE [Table 2]. The results of larvicidal and anthelmintic activities of ZOUE and ZOPUE are shown in Tables 3 and 4, respectively.

CONCLUSION

In the present research study, we evaluated antimicrobial activity of ZO using three bacterial strains, namely, *E. coli*, *S. aureus*, and *B. subtilis* and two fungal strains, namely, *A. flavus* and *A. niger*. Both ZOUE and ZOPUE at similar concentration showed bactericidal activity. Larvicidal activity of ZOPUE was found to be better when compared with ZOUE. Anthelmintic activity of ZOPUE was found to show better paralysis and death rate at a minimum concentration as compared to ZOUE.

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